

Title: *Production of Human Parathyroid Hormone*

*From Microorganisms*

Inventor(s): Kaare M. GAUTVIK, *et al.*

Appl. No.: 09/668,154

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**FIG. 1**

DNA SEQUENCE FOR HUMAN  
PREPROPARATHYROID HORMONE

10                   30                   50  
ATGATHCCNGCNAARGAYATGGCNAARGTNAATGATHGTNATGYTNGCNATHTGYTTYYTN

70                   90                   110  
ACNAARWSNGAYGGNAARWSNGTNAARAARMGNWSNGTWSNGARATHCARYTNATGCAY

130                  150                  170  
AAYYTNGGNAARCAYYTNAAYWSNATGGARMGNGTNGARTGGYTNMGNAARAARYTNCAR

190                  210                  230  
GAYGTNCAYAAYTTYGTNGCNYTNGGNGCNCNYTNGCNCNMNGAYGCNGGNWSNCAR

250                  270                  290  
MGNCCNMGNAARAARGARGAYAAYGTYTNGTNGARWSNCAYGARAARWSNYTNGGNGAR

310                  330  
GCNGAYAARGCNGAYGTNAAYGTYTNAACNAARGCNAARWSNCARTRR

M = A OR C

R = A OR G

W = A OR T

S = C OR G

Y = C OR T

H = A OR C OR T

N = A OR G OR C OR T

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**FIG. 2**

DNA SEQUENCE FOR HUMAN  
PREPROPARATHYROID HORMONE IN PLASMID pSSHPTH-10

10                   30                   50  
TATGATGATACTGC~~AAAAGACATGGCTAAAGTT~~TATGATTGT~~CATGTTGGCAATTGTT~~

70                   90                   110  
TCTTACAAAATCGGATGGAAATCTGTTAAGAAGAGATCGTGGAGT~~GAAAATACAGCTTAT~~

130                  150                  170  
GCATAACCTGGAAAACATCTGA~~ACTCGATGGAGAGAGTAGAATGGCTGC~~GTAAAGAGCT

190                  210                  230  
GCAGGATGTGCACAATT~~TTGTCCTGGAGCTCCTCTAGCTCCCAGAGATGCTGGTC~~

250                  270                  290  
CCAGAGGCCCGAAAAAAGGAAGACAATGTCTTGGTTGAGAGCCAT~~GAAAAGTCTTGG~~A

310                  330  
GAGGCAGACAAAGCTGA~~TGTAACTAAAGCTAAATCCCAGTGA~~

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**FIG. 3**

PORTION OF DNA SEQUENCE OF THE PLASMID  
FOR INSERTION INTO E. COLI, CODING FOR HUMAN  
PREPROPARATHYROID HORMONE WITH FLANKING SEQUENCES.

10                   30                   50  
TATGATGATHCCNGCNAARGAYATGGCNAARGTNATGATHGTNATGYTNGCNAHTGYTT

70                   90                   110  
YYTNACNAARWSNGAYGGNAAFWSNGTNAARAARMGNWSNGTNWSNGARATHCARYTNAT

130                  150                  170  
GCAYAAYYTNGGNAARCAYYTNAAYWSNATGGARMGNGTNGARTGGYTNMGNAARAARYT

190                  210                  230  
NCARGAYGTNCAYAAYTTYGTCNYTNGGNGCNCNYTNGCNCNCNGAYGCNSGNWS

250                  270                  290  
NCARMGNCCNMGNAARAARGARGAYAAYGTYNTNGTNGARWSNCAYGARAARWSNYTNGG

310                  330                  350  
NGARGCNGAYAARGCNGAYGTNAAYGTYTNACNAARGCNAARWSNCARTRRAATGAAA

370                  390                  410  
ACAGATATTGTCAGAGTTCTGCTCTAGACAGTGTAAGGGCAACAATACTGCTGCTAATTG

430  
AAAGCTCTATTA

M = A OR C

R = A OR G

W = A OR T

S = C OR T

Y = C OR T

H = A OR C OR T

N = A OR G OR C OR T

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**FIG. 4**

DNA SEQUENCE FOR HUMAN PREPROPARATHYROID  
HORMONE IN PLASMID pSSHPTH-10 WITH FLANKING SEQUENCES

10                   30                   50  
TATGATGATACTGCAAAAGACATGGCTAAAGTTATGATTGTATGTTGCAATTGTTT

70                   90                   110  
TCTTACAAAATCGGATGGAAATCTGTTAAGAAGAGATCTGTGAGTGAAATACAGCTTAT

130                  150                  170  
GCATAACTGGAAAACATCTGAACTCGATGGAGAGAGTAGAAATGGCTGCGTAAGAACCT

190                  210                  230  
GCAGGATGTGCACAATTGTTGCCCTGGAGCTCTAGCTCCCAGAGATGCTGGTC

250                  270                  290  
CCAGAGGCCCGAAAAAAGGAAGACAATGTCTGGTTGAGAGCCATGAAAAAGTCTTGG

310                  330                  350  
AGAGGCAGACAAAGCTGATGTGAATGTATTAACCTAAAGCTAAATCCCAGTGAAATGAAA

370                  390                  410  
ACAGATATTGTCAGAGTTCTGCTCTAGACAGTGTAGGGCAACAATACATGCTGCTAATTG

430  
AAAGCTCTATTA



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### FIG. 5

DNA SEQUENCE CODING FOR PREPROPARATHYROID HORMONE  
IN pSSHPTH-10 WITH FLANKING SEQUENCES, SHOWING  
THE CORRESPONDING AMINO ACID SEQUENCE OF  
PREPROPARATHYROID HORMONE

10                   30                   50  
TATGATGATACCTGCAAAAGACATGGCTAAAGTTATGATTGTATGTTGGCAATTGTTT  
MetIleProAlaLysAspMetAlaLysValMetIleValMetLeuAlaIleCysPh

70                   90                   110  
TCTTACAAAATCGGATGGGAAATCTGTTAAGAAGAGATCTGTGAGTGAAATACAGCTTAT  
eLeuThrLysSerAspGlyLysSerValLysLysArgSerValSerGluIleGlnLeuMe

130                 150                 170  
GCATAACCTGGAAAAACATCTGAACTCGATGGAGAGAGTAGAAATGGCTGCGTAAGAACGCT  
tHisAsnLeuGlyLysHisLeuAsnSerMetGluArgValGluTrpLeuArgLysLysLe

190                 210                 230  
GCAGGATGTGCACAATTTGTTGCCCTGGAGCTCCTCTAGCTCCCAGAGATGCTGGTTC  
uGlnAspValHisAsnPheValAlaLeuGlyAlaProLeuAlaProArgAspAlaGlySe

250                 270                 290  
CCAGAGGCCCGAAAAAAGGAAGACAATGTCTGGTTGAGAGCCATGAAAAAGTCTTGG  
rGlnArgProArgLysLysGluAspAsnValLeuValGluSerHisGluLysSerLeuG

310                 330                 350  
AGAGGCAGACAAAGCTGATGTGAATGTATTAACTAAAGCTAAATCCCAGTGGAAATGAAA  
yGluAlaAspLysAlaAspValAsnValLeuThrLysAlaLysSerGlnEnd

370                 390                 410  
ACAGATATTGTCAGAGTTCTGCTCTAGACAGTGTAGGGCAACAATACATGCTGCTAATTG

430  
AAAGCTCTATTA

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## FIG. 6

NUCLEOTIDE SEQUENCE OF THE MF 1-HPTH FISION GENE FROM pS LX5-HPTH1.

NUCLEOTIDE NOS. 1-173 MAKEUP THE MH 1 PROMOTER REGION AND 5' NONCODING SEQUENCE. 174-440 IS THE MF 1 N-TERMINAL CODING SEQUENCE. 441-695 IS THE HPTH SEQUENCE OBTAINED FROM pSSHPTH-10. 696-726 IS AN HPTH 3' NONCODING SEQUENCE FROM pSSHPTH-10. 727-732 IS FROM pUC19. 733-874 IS MF 1 3' NONCODING SEQUENCE AND TRANSCRIPTIONAL TERMINATION SIGNAL.

|   |     |     |
|---|-----|-----|
| 10  | 30  | 50  |
| AGTGCAAGAAAACCAAAAGCAACAACAGGTTGGATAAGTACATATATAAGAGGCCT      |     |     |
| 70  | 90  | 110 |
| TTGTTCATCAAAATGTTACTGTTCTACGATTACGATTCAAGAATAGTTCA            |     |     |
| 130   | 150 | 170 |
| AACAAGAAGATTACAAACTATCAATTCTACACAAATATAAACGACCAAAAGAACATGAGAT |     |     |
| 190   | 210 | 230 |
| TTCCTCAATTTTACTGCAGTTTATTCGCAGCATCCTCCGCATTAGCTGCTCCAGTCA     |     |     |
| 250   | 270 | 290 |
| ACACTACAACAGAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTATCGGTTACTCAG   |     |     |
| 310   | 330 | 350 |
| ATTTAGAAGGGATTTCGATGTTGCTGTTGCCATTTCACAGCACAAATAACGGGT        |     |     |
| 370   | 390 | 410 |
| TATTGTTATAAAACTACTATTGCCAGCATGCTGCTAAAGAAGAAGGGTATCTTGG       |     |     |
| 430   | 450 | 470 |
| ATAAAAGAGAGGCTGAAGCTCTGTGAGTGAAATACAGCTTATGCATAACCTGGAAAAC    |     |     |
| 490   | 510 | 530 |
| ATCTGAACTCGATGGAGAGTAGAATGGCTCGTAAGAAGCTGCAGGATGTGCACAATT     |     |     |
| 550   | 570 | 590 |
| TTGTTGCCCTGGAGCTCCTCTAGCTCCAGAGATGCTGGTCCCAGAGGCCCGAAAAA      |     |     |
| 610   | 630 | 650 |
| AGGAAGACAATGTCTGGTTGAGAGCCATGAAAAAGTCTGGAGAGGCAGACAAAGCTG     |     |     |
| 670   | 690 | 710 |
| ATGTGAATGTATTAACAAAGCTAAATCCCAGTGAAAATGAAAACAGATATTGTCAGAGT   |     |     |
| 730   | 750 | 770 |
| TCTGCTCTAGAGTCGACTTGTCCCAGTGACTTTAGCTCGTACAAATACAATATAC       |     |     |
| 790   | 810 | 830 |
| TTTCATTCCTCGTAAACACCTGTTCCATGTAATATCCTTCTATTTCTATTTCTT        |     |     |
| 850   | 870 |     |
| CGTTACCAACTTACACATACTTATAGCTAT                                |     |     |

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**FIG. 7**

PARTIAL DNA SEQUENCE FOR THE PLASMID FOR INSERTION INTO ~~TEG~~ CENTER 1600/2900  
IN WHICH: NUCLEOTIDE NOS. 1-173 MAKEUP THE MF 1 PROMOTER  
REGION AND 5' NONCODING SEQUENCE. 174-440 IS THE MF 1 N-TERMINAL  
CODING SEQUENCE. 441-695 IS AN HPTH SEQUENCE. 696-726 IS AN  
HPTH 3' NONCODING SEQUENCE FROM pSSHPTH-10. 727-732 IF FROM  
pUC19. 733-874 IS MF 1 3' NONCODING SEQUENCE AND TRANSCRIPTIONAL  
TERMINATION SIGNAL.

|  |     |     |
|--|-----|-----|
| 10   |     | 30  |
| AGTGCAAGAAAACCAAAAGCAACAACAGGTTGGATAAGTACATATATAAGAGGGCCT      |     |     |
| 70   | 90  | 110 |
| TTTGTCCCATCAAAAATGTTACTGTTACGATTCAATTACGATTCAAGAATAGTTCA       |     |     |
| 130  | 150 | 170 |
| AACAAGAAGATTACAAACTATCAATTACATACACAATATAAACGACCAAAAGAACATGAGAT |     |     |
| 190  | 210 | 230 |
| TTCCTTCAATTTCAGTTACTGCAGTTTATTGCAGCATCCTCCGCATTAGCTGCTCCAGTCA  |     |     |
| 250  | 270 | 290 |
| ACACTACAAACAGAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTATCGGTTACTCAG   |     |     |
| 310  | 330 | 350 |
| ATTAGAAGGGGATTCGATGTTGCTGTTTGCATTTCAACAGCACAAATAACGGGT         |     |     |
| 370  | 390 | 410 |
| TATTGTTATAAATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTTGG     |     |     |
| 430  | 450 | 470 |
| ATAAAAGAGAGGCTGAAGCTWSNGTNWSNGARATHCARYTNATGCAYAAYYTNGGNAARC   |     |     |
| 490  | 510 | 530 |
| AYYNAAYWSNATGGARMGNGTNGARTGGYTNMGNAARAARYTNCARAYGTNCAYAAYT     |     |     |
| 550  | 570 | 590 |
| TYGTNGCNYTNGGNGNCNYTNGCNCCNMGNAYGCNGGNWSNCARMGNCCNMGNAA        |     |     |
| 610  | 630 | 650 |
| ARGARGAYAAYGTNYTNGTNGARWSNCAYGARAARWSNYTNGGNGARGCNGAYAARGCNG   |     |     |
| 670  | 690 | 710 |
| AYGTNAAYGTNYTNACNAARGCNAARWSNCARTRRAATGAAAACAGATATTGTCAGAGT    |     |     |
| 730  | 750 | 770 |
| TCTGCTCTAGAGTCGACTTGTCCACTGTACTTTAGCTCGTACAAAATACAATATAC       |     |     |
| 790  | 810 | 830 |
| TTTCATTCTCCGTAAACACCTGTTTCCCATGTAATATCCTTTCTATTTCGTTT          |     |     |
| 850  | 870 |     |
| CGTTACCAACTTACACATACTTATATAGCTAT, WHEREIN                      |     |     |

M = A OR C

R = A OR G

W = A OR T

S = C OR G

Y = C OR T

H = A OR C OR T

N = A OR G OR C OR T

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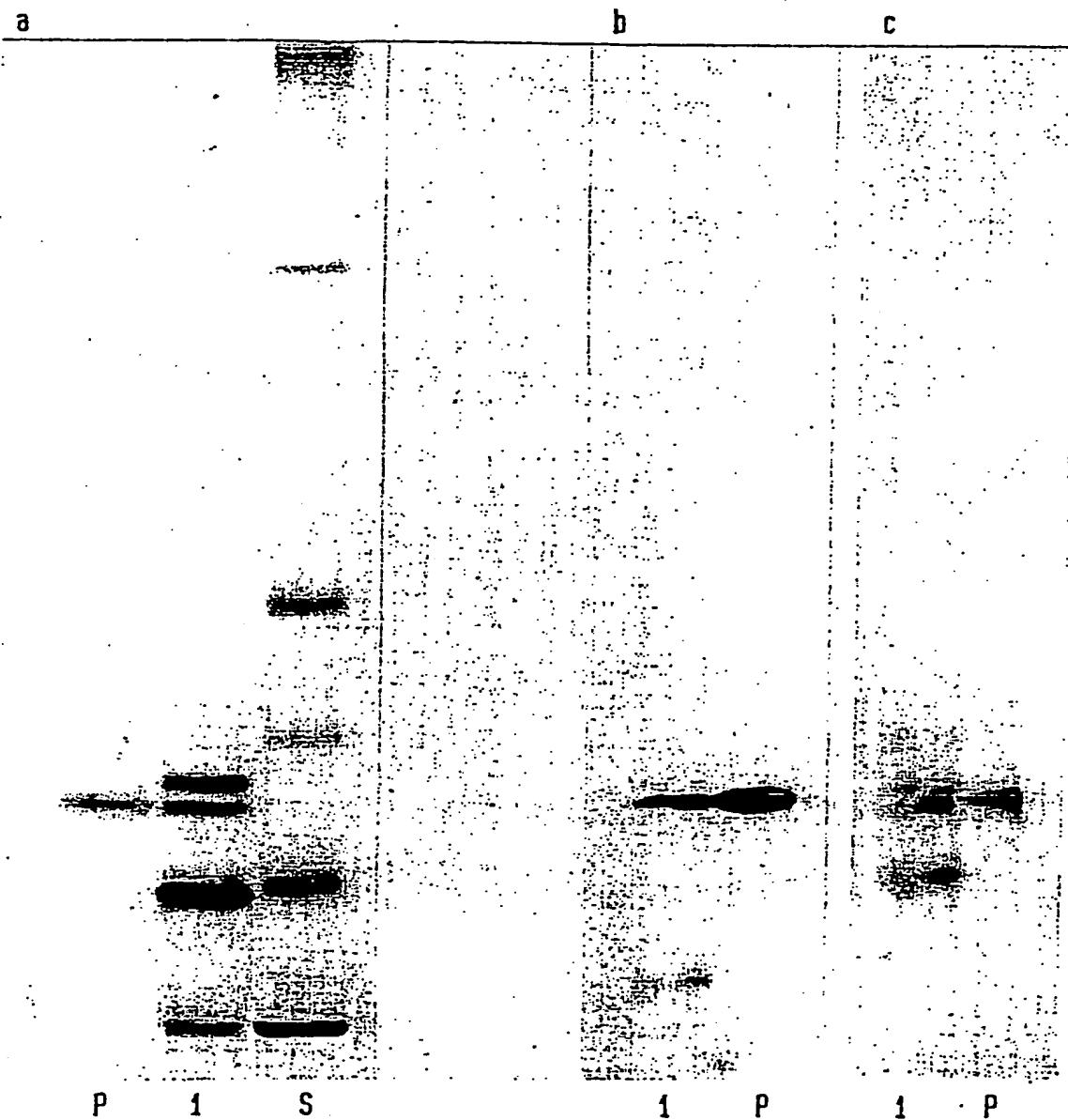
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FIG. 8



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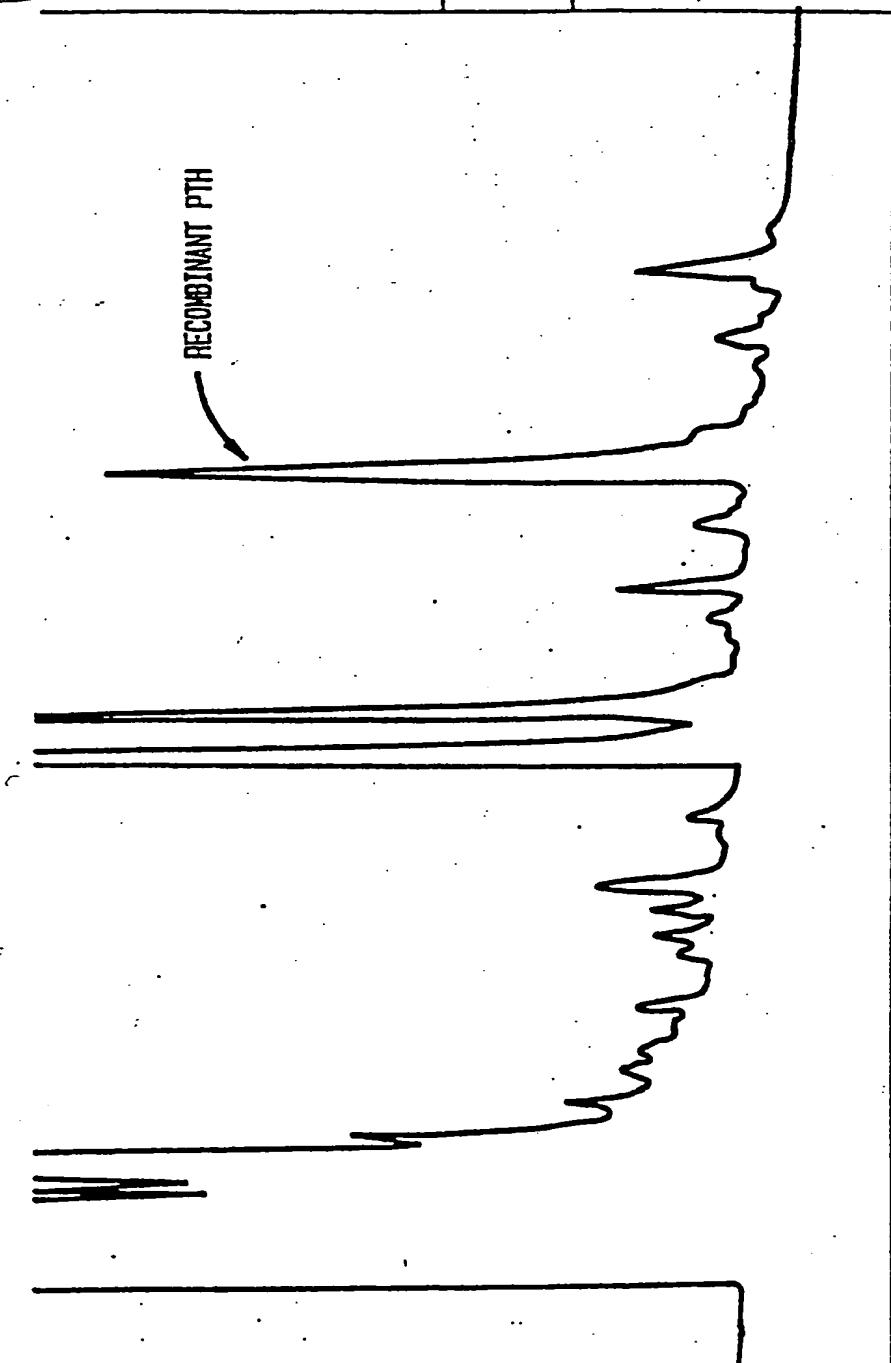


ACN [W]

-60

-35

FIG. 9A



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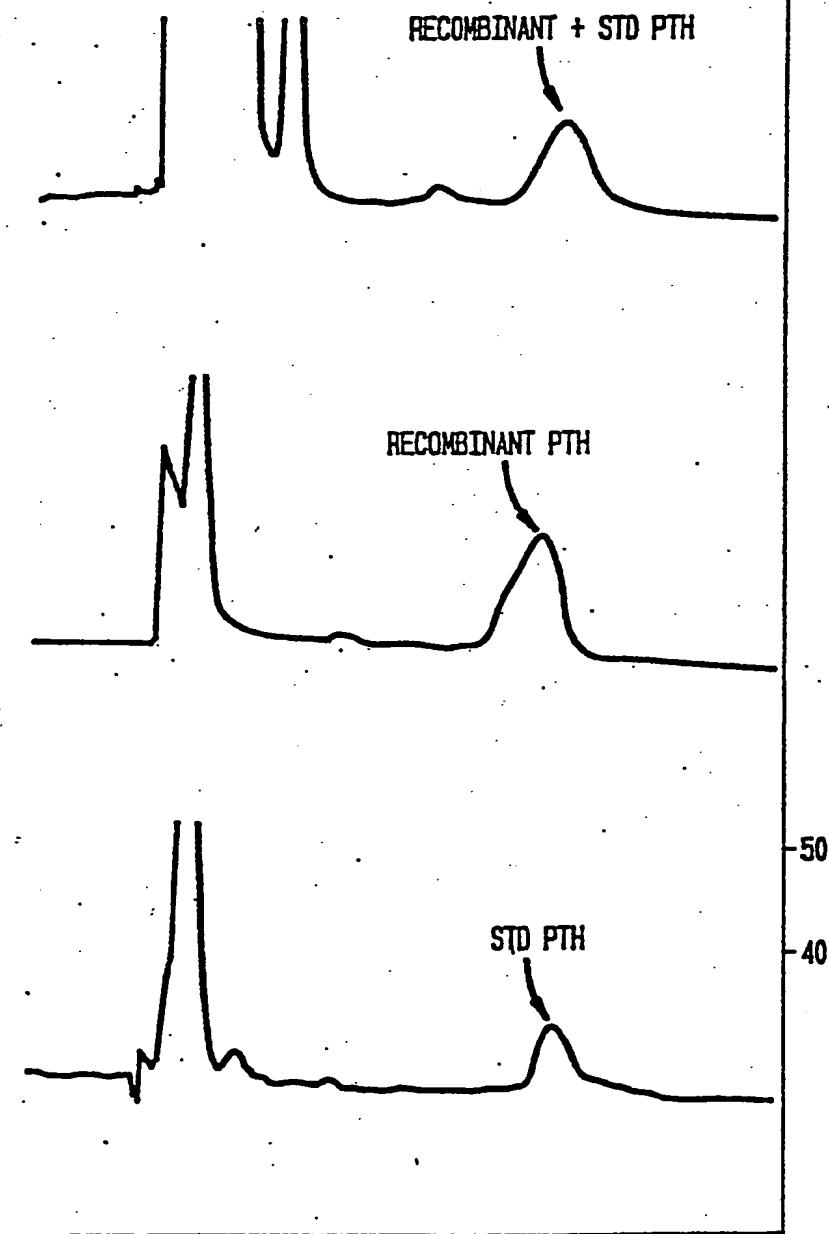
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**FIG. 9B**

AcN (x)



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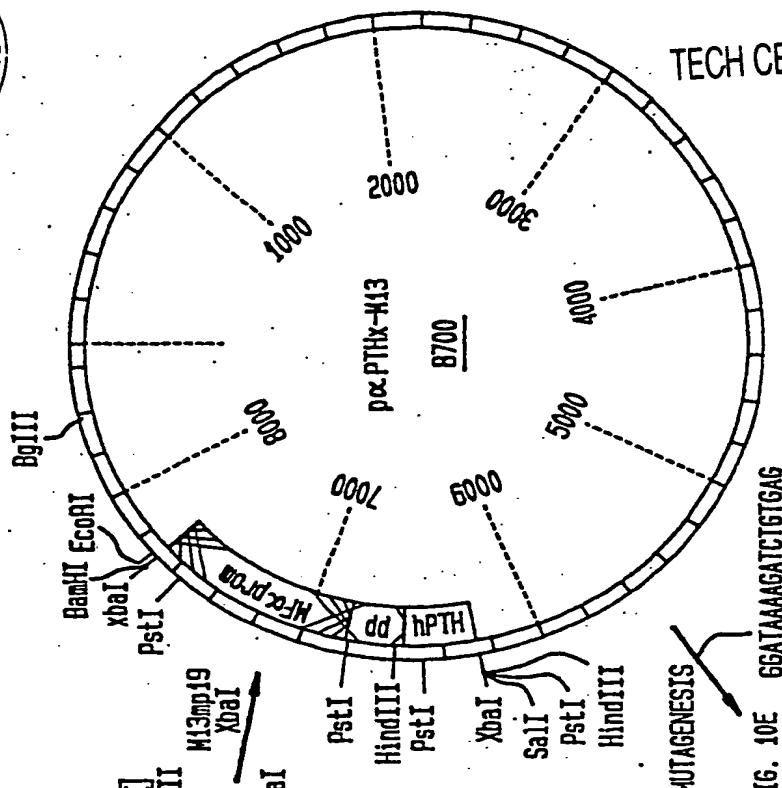
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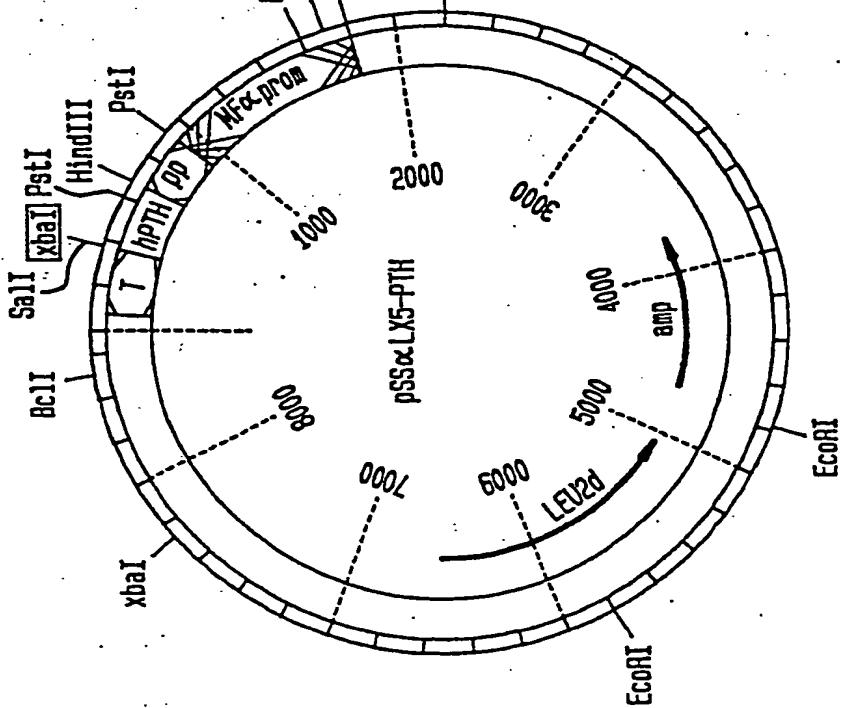
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**FIG. 10B**



**FIG. 10A**



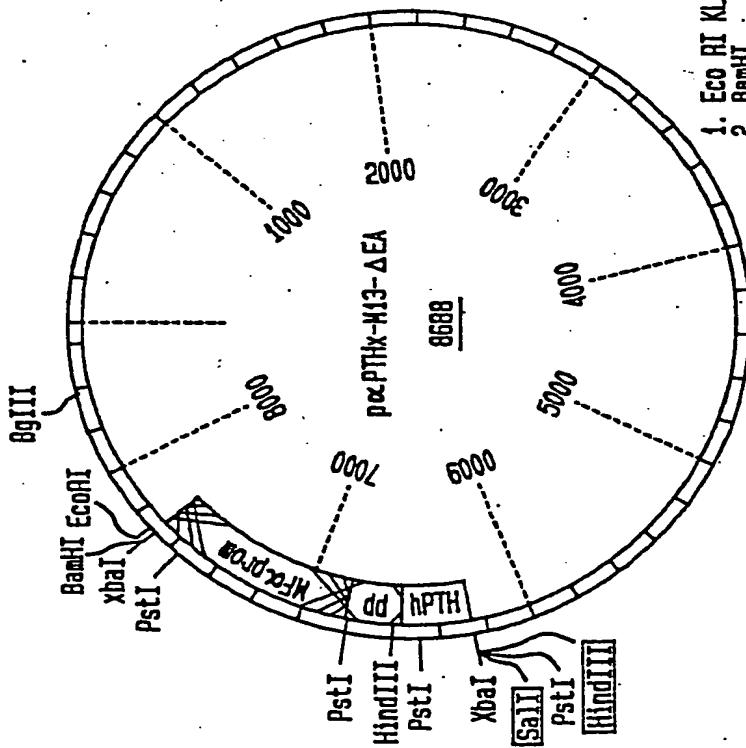
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**FIG. 10C**

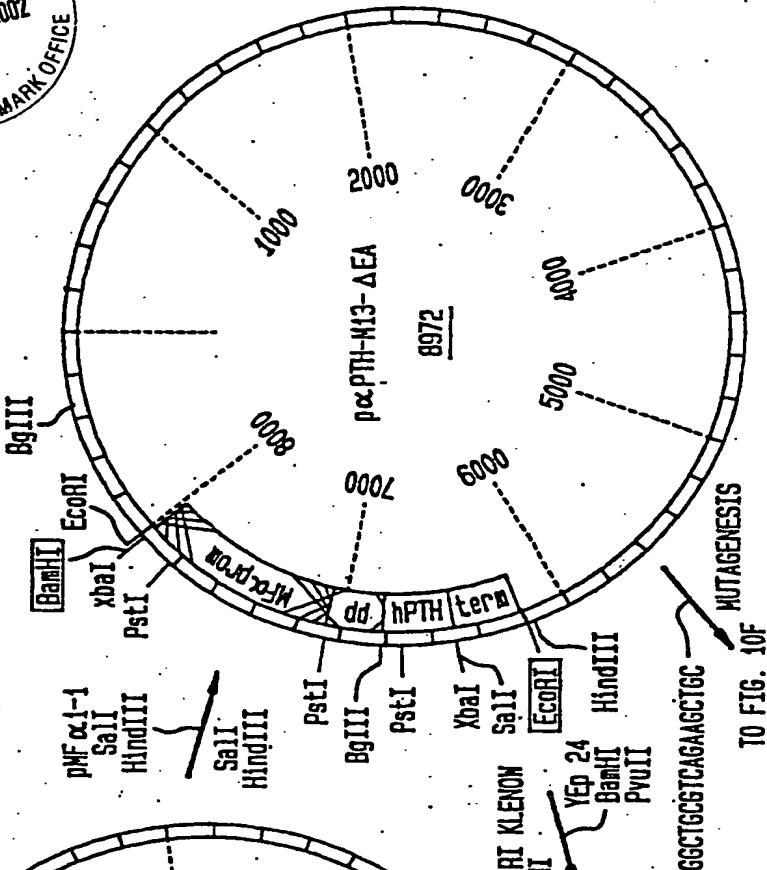


TO FIG. 10E  
1. Eco RI KLENOW  
2. BamHI

GGCTGGGTCAAGCTGC

TO FIG. 10F

**FIG. 10D**



MUTAGENESIS

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FIG. 10F

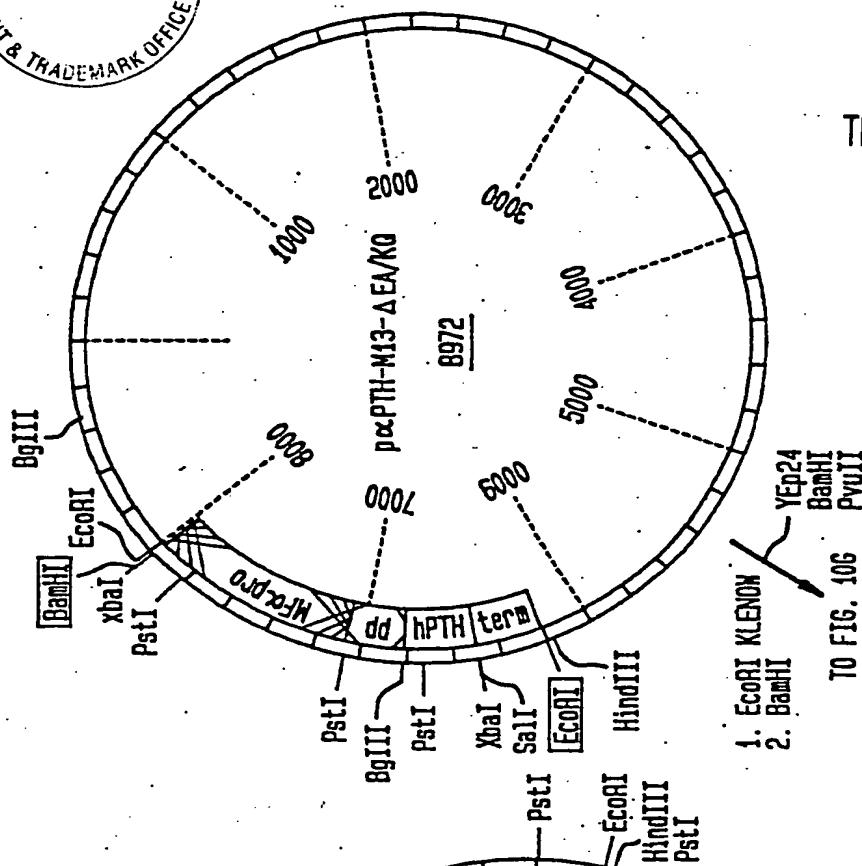
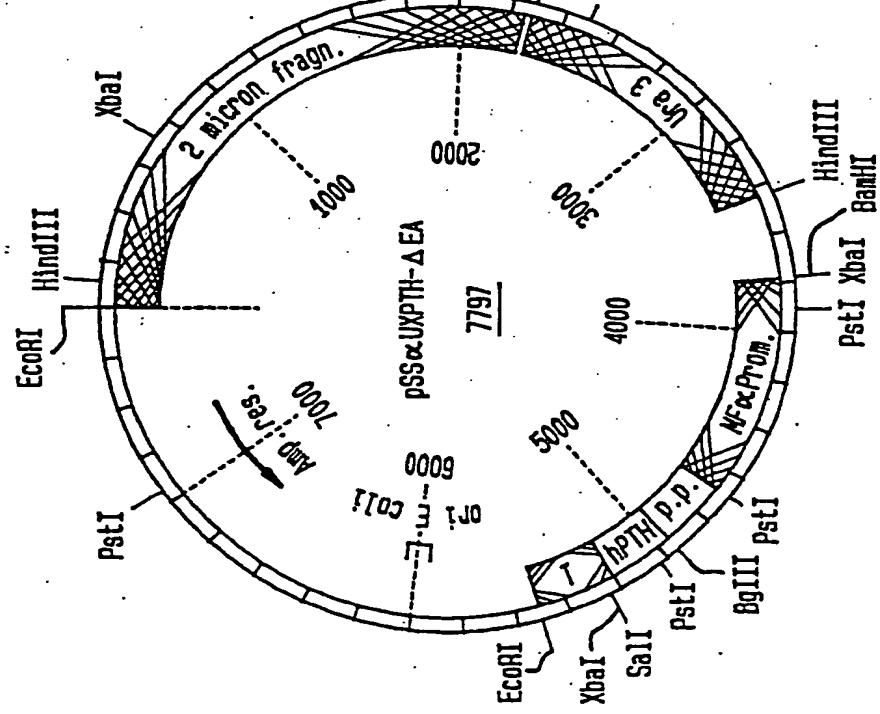


FIG. 10E



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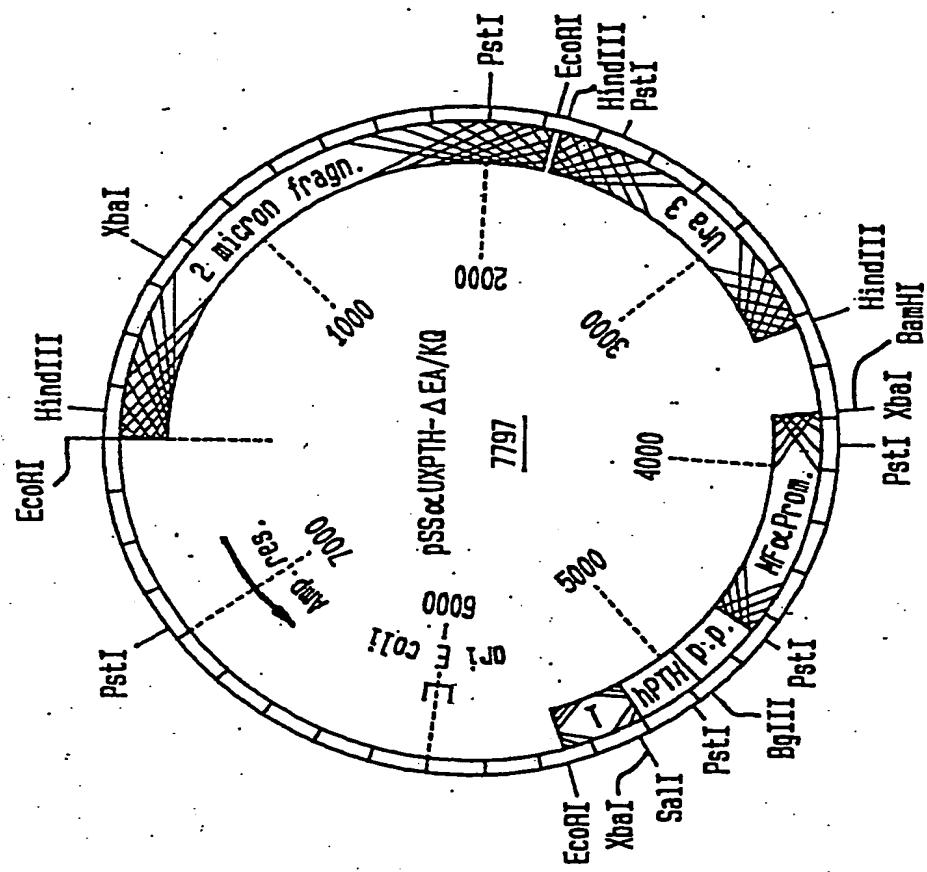


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FIG. 10G



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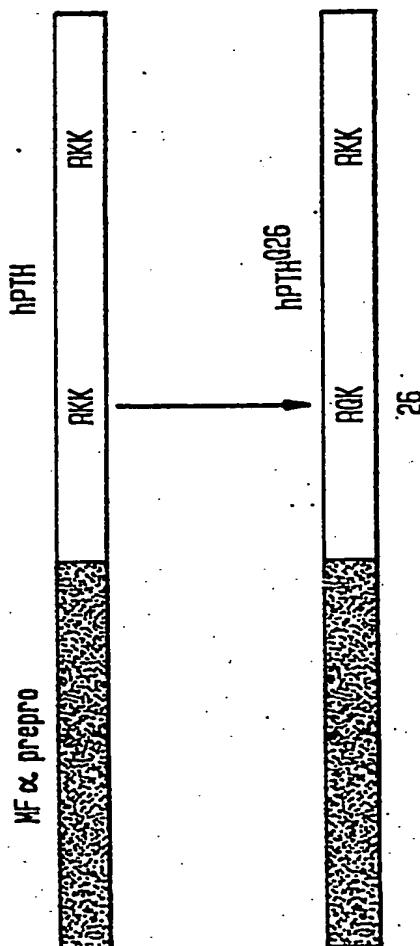
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FIG. 11

IN VITRO MUTAGENESIS OF hPTH



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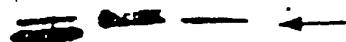


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FIG. 12



1 2 M

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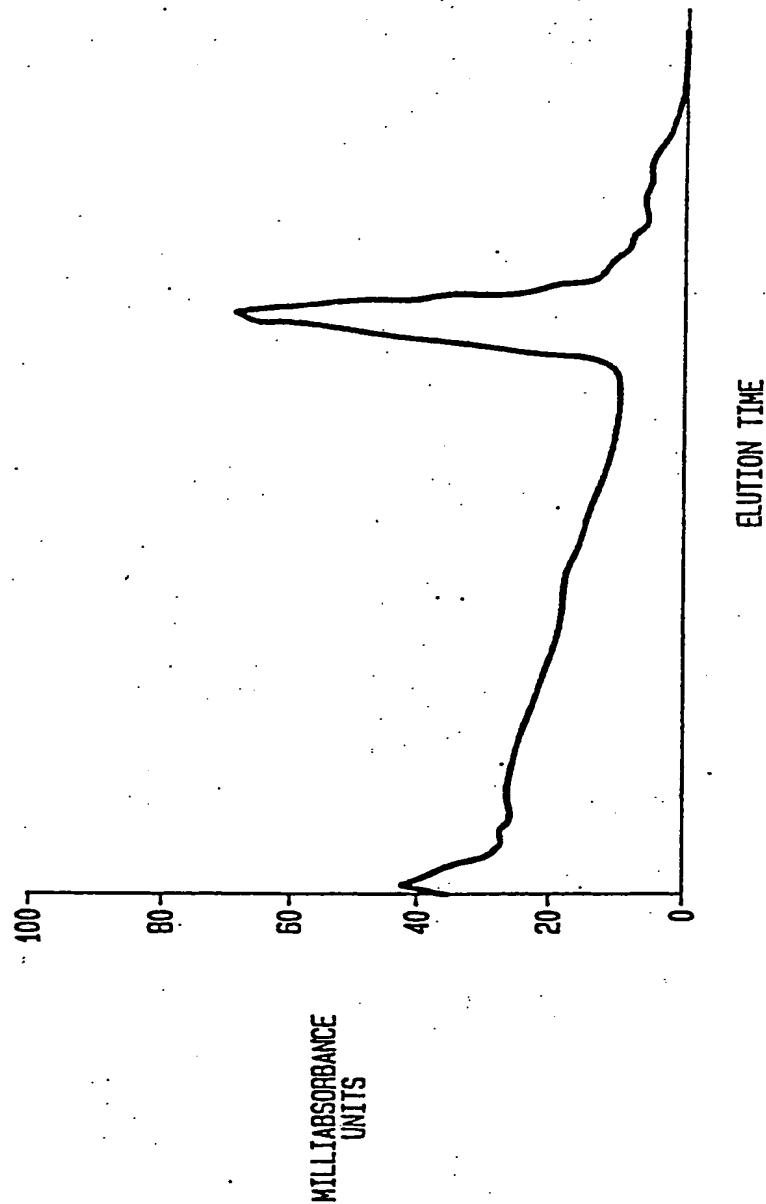
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FIG. 13A  
HPLC CHROMATOGRAM OF hPTH (026)



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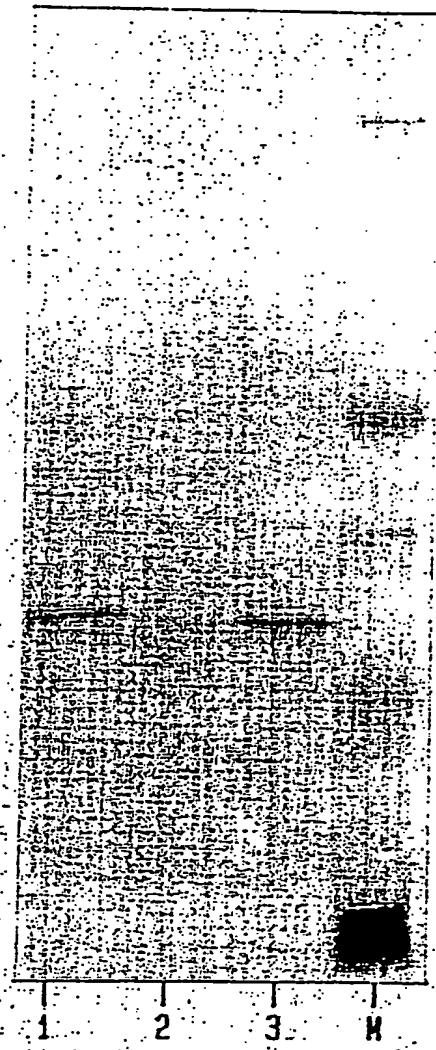


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FIG. 13B



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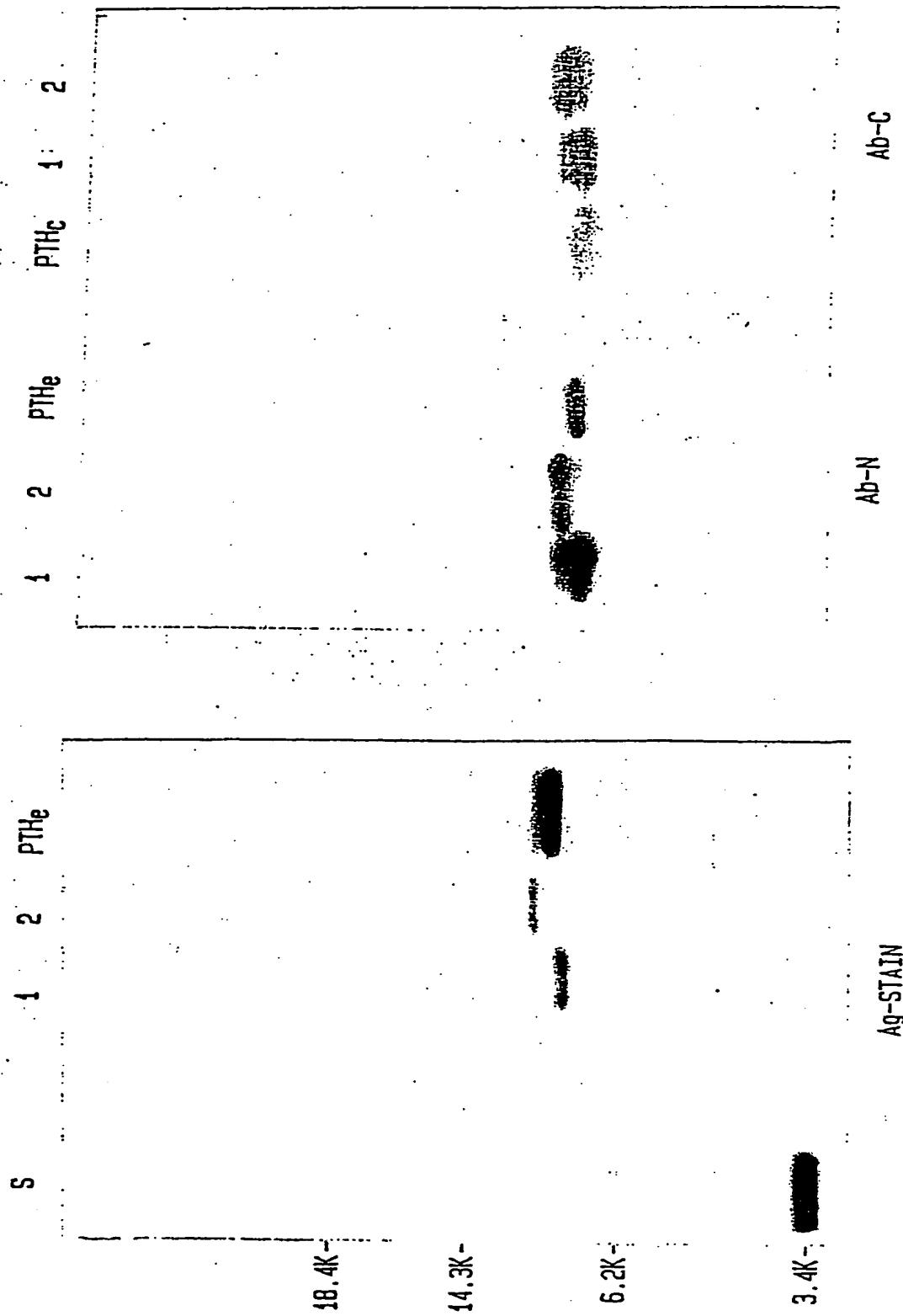
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FIG. 14



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FIG. 15

